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Aerobic and anaerobic biosynthesis of nano-selenium for remediation of mercury contaminated soil

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Running title: Biosynthesis of nano-Se^0 and mercury remediation

ABSTRACT

Selenium (Se) nanoparticles are often synthesized by anaerobes. However, anaerobic bacteria cannot be directly applied for bioremediation of contaminated top soil which is generally aerobic. In this study, a selenite-reducing bacterium, *Citrobacter freundii* Y9, demonstrated high selenite reducing power and produced elemental nano-selenium nanoparticles (nano-Se^0) under both aerobic and anaerobic conditions. The biogenic nano-Se^0 converted 45.8-57.1% and 39.1-48.6% of elemental mercury (Hg^0) in the contaminated soil to insoluble mercuric selenide (HgSe) under anaerobic and aerobic conditions, respectively. Addition of sodium dodecyl sulfonate enhanced Hg^0 remediation, probably owing to the release of intracellular nano-Se^0 from the bacterial cells for Hg fixation. The reaction product after remediation was identified as non-reactive HgSe that was formed by amalgamation of nano-Se^0 and Hg^0. Biosynthesis of nano-Se^0 both aerobically and anaerobically therefore provides a versatile and cost-effective remediation approach for Hg^0-contaminated surface and subsurface soils, where the redox potential often changes dramatically.

Keywords: Bioremediation; selenium; mercury; metal immobilization; selenium nanoparticles

1. Introduction

Mercury (Hg) is a naturally occurring non-essential highly toxic metal in the Earth's crust, and it is widely used in many industries such as the extraction of gold from ores, production of NaOH and chlorine in the chlor-alkali industry, and manufacture of compact fluorescent lamps, cosmetics, insecticides and herbicides (Boening, 2000). In some cases, improper use has led to extensive mercury pollution of soil. For example, mercury concentrations in the soil around a chlor-alkali plant in the Netherlands reached up to 1150 mg kg^{-1} (Bernaus et al., 2006). Mercury emissions were also detected in surrounding soils and sealed waste ponds near a chlor-alkali factory (Southworth et al., 2004).

Mercury speciation in contaminated soil can be classified into water soluble, elemental, exchangeable, strongly-bound, organic, sulfide and residual fractions. Normally, elemental mercury comprises a small proportion of the total mercury in soil whereas in mercury or gold mining regions and in chlor-alkali plant soil, elemental mercury may account for a much larger part of the total mercury. In the Idrija mercury mine region, Slovenia, HgS is the predominant mercury fraction, followed by Hg^0 (Kocman et al., 2004). Elemental mercury accounted for ~95% of the total mercury in soils heavily contaminated with mercury in Venezuela (García-Sánchez et al., 2006). Soil beneath and adjacent to the Pavlodar Chemical Plant in Kazakhstan was also contaminated by mercury, and ~88-98% of the total mercury can be present as elemental mercury (Neculita et al., 2005). Therefore, there is an urgent need to treat

elemental mercury-contaminated soil, particularly that caused by the industries mentioned above.

Selenium (Se) is in the same group as sulfur in the Periodic Table, and has an extremely high affinity for mercury with $\triangle G^0$= -38.1 kJ mol^{-1}, which is higher than that for sulfur (Ho et al., 2015). A large amount of work has been carried out on detection of mercury and selenium in fish, marine mammals and humans. The molar ratio of mercury to selenium in such samples was approximately 1, which suggested detoxification of mercury into less toxic mercuric selenide (HgSe) (Southworth et al., 2000; Squadrone et al, 2015). Selenium nanoparticles have already been shown to be effective for mercury removal from off gases, and unstabilized amorphous nano-Se^0 showed a strong mercury capture capacity of 188 mg g^{-1} dry weight (Johnson et al., 2008; Lee et al., 2009). Biogenic red amorphous nano-Se^0 has also been applied to sequester mercury vapour released from mercury-contaminated museum specimens, the historic mercuric chloride treatment to preserve specimens leading to mercury volatilization (Fellowes et al., 2011). Nano-Se^0 therefore appears to be a promising mercury-trapping agent for cleanup, disposal, recycling and packaging applications (Ralston, 2008).

Most of these examples of mercury removal by selenium are concerned with mercury vapour in the atmosphere. However, this technique can also be applied to the aquatic environment. For example, *Pseudomonas fluorescens* could reduce SeO_3^{2-} and Hg^{2+}

into elemental forms, the interaction between these two elements resulting in the formation of Hg-Se complexes within the cells with a Hg:Se molar ratio close to 1 (Yang et al., 2011). Bioreduced Hg^0 by a strain of *Shewanella putrefaciens* was captured as HgSe by extracellular biogenic amorphous selenium nanospheres (Jiang et al., 2012). However, no studies have been carried out which have tested the capacity of biogenic nano-Se^0 to immobilize mercury in soil.

Bioremediation of contaminated soil can be limited by the redox potential and the performance of the remediating bacteria in aerobic and anaerobic conditions. Surface soil layers are usually aerobic while subsurface soil layers may be anoxic, which means that both aerobic and anaerobic processes may be required. In addition, the soil redox potential during bioremediation may change drastically as bacterial cultures and substrates are applied. This may increase the cost, complexity and performance of bioremediation. Therefore, an ability for microbes to produce nano-Se^0 both aerobically and anaerobically may be relevant for the bioremediation of mercury-contaminated soils. Using versatile facultative bacteria to remediate soils with quite different redox potentials could be simpler and more effective.

In the present study, the performance of the facultative anaerobe *Citrobacter freundii* Y9, which can produce amorphous nano-Se^0 under anaerobic and aerobic conditions, in sequestering elemental mercury in soil was evaluated. Sequential soil extraction of mercury was carried out to determine changes in mercury speciation, and the reaction

products were characterized by scanning electron microscopy with energy-dispersive X-ray spectrometry (SEM-EDS), X-ray diffraction (XRD), transmission electron microscopy (TEM) and X-ray photoelectron spectroscopy (XPS).

2. Materials and methods

2.1. Bacteriogenic nano-Se^0

Citrobacter freundii Y9, isolated from sludge from an anaerobic sulfate-reducing bioreactor in Urumqi, China was used in this study, and the sequence has been submitted to Gene Bank (number KF781347). The growth medium contained the following components: 1.0 g K_2HPO_4, 0.1 g $MgCl_2$, 0.2% yeast extract, 10 mM sodium citrate in 1 L Milli-Q water. The medium was adjusted to pH 7.0-7.2 using 0.1 M HCl, and sterilized in a vertical heating pressure stream (LDZX-75KBS, Shanghai, China). The bacteria were cultured at 26^0C in 500 ml serum bottles in a Whitley DG250 anaerobic workstation (Don Whitley Scientific, West Yorkshire, England), and aerobically in 250 ml flasks with constant shaking at 150 rpm.

To measure the selenite reduction activity of *C. freundii* Y9, late logarithmic phase cells (5%) were inoculated into fresh medium containing 1 mM sodium selenite, added from a sterile 500 mM sodium selenite stock solution. At appropriate time intervals, samples were collected and filtered using 0.22 μm hydrophilic polyestersulfone membranes. Selenite in the filtrates was analyzed by LC-HGAFS (Liquid Chromatography-Hydride Generation Atomic Fluorescence Spectrometry) (Jitian, Beijing, China). Determination of the number of viable cells (colony-forming units, CFU) was conducted as follows to measure the growth of bacteria (Tugarova et al, 2014). A series of consecutive ten-fold dilutions of bacterial suspensions were made using sterile physiological saline (0.87% NaCl); 200 μl of the corresponding

diluted samples were then spread on solid nutrient broth medium and cultured for 4-5 d at 26^0C. Abiotic nano-Se^0 was prepared using L-ascorbic acid as a reductant to reduce H_2SeO_3, polyvinyl alcohol (PVA, 0.05%) was used as a soft template. The abiotic nano-Se^0 was centrifuged at 10,000×g for 10 min and then re-suspended in PVA solution (0.05%). Biogenic and abiotic selenium were characterized by SEM-EDS and XRD.

2.2. Elemental mercury immobilization in soil

Biogenic and abiotic nano-Se^0 were used to capture mercury in contaminated soil under aerobic and anaerobic conditions. Soil was collected from farmland near Urumqi, China, sterilized in a vertical heating pressure stream (LDZX-75KBS, Shanghai, China), and air-dried, sieved (1 mm), and sterilized again under UV light irradiation for 2 h. Liquid mercury was added to the soil directly which was then aged for two months. The mercury immobilization tests were performed in centrifuge tubes which contained 25 g of elemental mercury contaminated soil and 25 ml medium containing 4 mM elemental selenium. When biogenic nano-Se^0 was used to treat mercury contaminated soil, one group contained 1% sodium dodecyl sulfate (SDS) to lyse the bacteria and release intracellular Se^0. The original concentration of soil mercury was analyzed using a mercury analyzer (Lumex RP91C, Saint Petersburg, Russia). After one week, the different mercury fractions in the soil samples were analyzed. A control without addition of nano-Se^0 was also treated in the same way. The elemental selenium in the medium or in the PVA suspensions was centrifuged at

12,000×g for 10 min and then the Se-free supernatant was added to the control. Anaerobic and aerobic immobilization were performed inside a Whitley DG250 anaerobic workstation or in a fume hood, respectively.

Sequential extraction procedures were used to evaluate mercury speciation in the soil, according to previously published methods (Biester and Scholz, 1996; Shi et al., 2005). Mercury compounds were classified into the following fractions: F1: total mercury; F2: elemental mercury; F3: water-soluble and exchangeable mercury; F4: mercury bound to organic matter; and F5: residual mercury. Total mercury (F1) was analyzed using a mercury analyzer (Lumex RP91C, Saint Petersburg, Russia) and this value was labelled THg1. For elemental mercury (F2), the soil was heated at 180^0C for 2 h in a muffle furnace to separate out the elemental mercury. After this treatment, the total mercury left in the soil was again analyzed and this value was labelled THg2. The remaining soil was set aside for the following treatment. For water-soluble mercury and exchangeable mercury (F3), 20 ml Milli-Q water (18 $M\Omega cm^{-1}$) was added to 2 g soil from the F2 treatment and shaken for 2 h. The mixture was then centrifuged for 20 min at 12,000×g. Another 20 ml of 1 M $CaCl_2$ (pH=5) was added to the soil and shaken for 2 h. The mixture was then centrifuged for 20 min at 12,000 ×g, then air dried. The total mercury left in the soil was again analyzed and this value was labelled THg3. The remaining soil was set aside for the following treatment. For mercury bound to organic matter (F4), 20 ml of 0.2 M NaOH was added to the treated soil from F3 and shaken for 2 h. The mixture was centrifuged as described previously,

and then 20 ml CH_3COOH 4% (v/v) was added to the soil and shaken for 2 h. The mixture was then centrifuged as previously described, air dried and the total mercury left in the soil analyzed as above, which was labelled THg4. According to the following formulae, the concentrations of the different mercury fractions in the soil were obtained: F1= THg1; F2= THg1-THg2; F3= THg2-THg3; F4= THg3-THg4; F5= THg4.

2.3. SEM-EDS, XRD, TEM and XPS analyses

The synthesized selenium particles and the soil after the experiments were analyzed by SEM-EDS. These samples were first freeze-dried in a vacuum freeze dryer (Labconco, Kansas, USA) then coated with gold with a sputter coater (Emitech K575, Kent, UK). Samples were examined using a scanning electron microscope (Zeiss Super 55VP, Oberkochen, Germany). Elemental analysis was carried out using energy-dispersive X-ray spectrometry (Bruker XFlash 5010, Berlin, Germany).

Samples for XRD were first freeze-dried, and then XRD spectra were obtained using an X-ray diffractometer (Bruker D8, Karlsruhe, Germany) with a Cu anode (40 kV and 30 mA) and scanning from 5 to 80^0 2θ.

In order to further characterize biogenic selenium particles, TEM was conducted as follows (Zhang and Frankenberger, 2006). Cells were harvested by centrifugation (10,000×g, 10 min) and fixed with 2.5% para-formaldehyde + 2.5% glutaraldehyde

in 0.1 M cacodylate buffer (pH 7.2) and post-fixed with 1% OsO_4 + 0.15% ruthenium red in 0.1 M cacodylate buffer. After washing three times with Milli-Q water, cells were dehydrated in graded acetone solutions (30, 50, 70, 90 and 100% for 15 min each time) and then embedded in Epon-Araldite. Blocks were sectioned using a Reichert Supernova Microtome (Leica AG, Wien, Austria) using a diamond knife producing sections approximately 80 nm in thickness. The samples were observed using a JEM-1200EX electron microscope (JEOL, Tokyo, Japan).

X-ray photoelectron spectroscopy was carried out on powders using a Thermo ESCALAB 250Xi spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) using an Al Ka monochromatized source. Surface charging effects were corrected with a C 1s peak at 284.6 eV as a reference. Curve fitting and decomposition were achieved assuming Gaussian-Lorentzian fitting following Shirly background subtraction.

2.4. Reagents

All the chemicals and reagents used in this study were of analytical grade. SDS, H_2SeO_3 and Na_2SeO_3 were supplied by Guang Fu (Tianjin, China). Selenite was prepared as a 500 mM stock solution in Milli-Q water (18 $M\Omega cm^{-1}$) and sterilized using 0.22 μm hydrophilic polyestersulfone membrane filters (Shanghai, China). Liquid mercury was obtained from Sinopharm Chemical Reagent (Shanghai, China). PVA was obtained from Sigma-Aldrich Ltd. L-ascorbic acid was supplied by Yong

227 Sheng (Tianjin, China).

228

229 *2.5. Statistical analysis*

230 The size of selenium particles was calculated using Image-Pro Plus 6.0 based on SEM

231 spectra. All experiments were carried out in triplicate; error bars on figures show

232 standard deviations'.

233

3. Results and discussion

3.1. Bacteriogenic nano-Se^0

C. freundii Y9 could reduce selenite to elemental selenium particles, which was evident by the colour of the medium changing to red/orange, under both aerobic and anaerobic treatments. There was no colour change and no change of selenite concentration in the abiotic control which demonstrated that it was the presence of growing bacteria that led to the reduction of selenite. After inoculation, bacterial growth was concomitant with the process of reduction. *C. freundii* Y9 showed more tolerance to selenite under anaerobic conditions and over 24 h, the medium turned red, and there was a rapid decrease in selenite concentration with complete removal after 5 d (Fig. 1). However, under aerobic conditions the medium turned a weaker red after 24 h with the efficiency of selenite reduction being 27% after 5 d incubation, the concentration of selenite remaining stable after this time (Fig. 1). Anaerobic selenite reduction was rapid and more pronounced than in aerobic conditions. In an anaerobic mode of respiration, selenite can be used as an electron acceptor in dissimilatory reduction (Macy et al., 1989), or be reduced and incorporated into organic compounds in assimilatory reduction (Lortie et al., 1992; Gadd, 1993). However, the mechanisms under aerobic conditions are not clearly understood.

SEM of *C. freundii* Y9 showed that particles were present inside the cells after exposure to 1 mM selenite; such particles were also detected extracellularly (Fig. 2b, c, d). EDS spectra of the particles confirmed the presence of selenium with characteristic

256 selenium absorption peaks at 1.37 and 11.22 keV (Fig. 2e). The calculated diameter of
257 selenium particles ranged from 200-800 nm, with the average diameter being 580±
258 109 nm. XRD patterns showed a broad peak at 2θ values from 25^0 to 30^0, which
259 indicated that the selenium particles formed were amorphous in nature (Fig. 2f). TEM
260 showed that electron-dense particles were present inside the cells near the cytoplasmic
261 membrane after incubation with 1 mM selenite (Fig. 2g, h). The biogenic selenium
262 was deposited inside the cells or extracellularly and during cell lysis the elemental
263 selenium could be released into the extracellular medium.
264
265 *C. freundii* is commonly found in soil, freshwater and marine habitats. Although,
266 selenate reduction has been reported in *C. freundii* (Zhang et al., 2008), there is less
267 work on selenite reduction, and the electron transfer system is different between
268 selenate and selenite reduction in this organism (Siddique et al., 2006). As selenate is
269 generally more toxic than selenite (Hockin and Gadd, 2003, 2006), it is perhaps better
270 to use selenite as an electron acceptor to obtain nano-Se^0.
271
272 *3.2. Abiotic nano-Se^0*
273 The mixture of PVA-stabilized selenium nanoparticles had a red/orange colour and
274 remained stable on prolonged incubation. SEM and EDS spectra confirmed the
275 presence of elemental nano-Se^0 (Fig. 3). The diameters of these nano-Se^0 particles
276 ranged from 10-90 nm with an average value of 71±16 nm. However, without PVA in
277 solution, a dark red precipitate of Se^0 appeared. The XRD pattern of

chemically-reduced elemental selenium was the same as that for biogenic elemental selenium, indicating that the elemental selenium formed here was also amorphous.

3.3. Elemental mercury immobilization in soil

The ability of biogenic and abiotic elemental amorphous nano-Se^0 to immobilize Hg^0 in soil was comparatively studied. The total mercury in the contaminated soil was 21.43±2.51 μg g dry $weight^{-1}$ which is ~350-fold higher than values commonly found. Elemental mercury was the primary fraction (17.63±2.10 μg g^{-1} dry weight) which accounted for 78.2-84.6% of the total mercury, while 11.1-11.7% (2.46±0.28 μg g^{-1}) of the mercury occurred in the insoluble residual mercury fraction. When mercury-contaminated soil was supplied with nano-Se^0, the total mercury decreased only slightly, some possibly being volatilized or adhering to surfaces of the bioreactor. However, the mercury speciation changed significantly, especially in the elemental and residual fractions (Fig. 4). Under anaerobic conditions, Hg^0 present in the mercury-contaminated control decreased by 11.3% (1.99 μg g^{-1}). However, there was a 73.5% (12.96 μg g^{-1}) and 63.5% (11.20 μg g^{-1}) decrease in Hg^0 when the soil was supplied with biogenic Se^0 + SDS and biogenic Se^0, respectively. The SDS was used in an attempt to release intracellular Se^0 from the bacterial cells and therefore enhance the reaction between Hg^0 and Se^0. For the abiotic Se^0 treatment, 49.2% (8.68 μg g^{-1}) of the Hg^0 fraction decreased. Under aerobic conditions, Hg^0 present in the control decreased by 12.5% (2.67 μg g^{-1}). However, there was a 65.8% (11.60 μg g^{-1}) and 61.25% (10.79 μg g^{-1}) decrease in Hg^0 when the soil was supplied with biogenic Se^0 +

SDS and biogenic Se^0, respectively. For the abiotic aerobic Se^0 treatment, Hg^0 decreased by 38.9% (6.87 μg g^{-1}). Concomitant with the decrease in Hg^0, the residual mercury fraction was found to increase significantly. Under anaerobic conditions, residual mercury in the mercury-contaminated control increased by 0.73 μg g^{-1}. However, there was a 10.79 μg g^{-1} and 8.80 μg g^{-1} increase in residual mercury when the soil was supplied with biogenic Se^0 + SDS and biogenic Se^0, respectively. For the abiotic Se^0 treatment, residual mercury increased by 4.44 μg g^{-1}. Under aerobic conditions, residual mercury in the control increased by 0.85 μg g^{-1}. However, there was a 9.41 μg g^{-1} and 7.75 μg g^{-1} increase in residual mercury when the soil was supplied with biogenic Se^0 + SDS and biogenic Se^0, respectively. For the abiotic aerobic Se^0 treatment, residual mercury was increased by 3.15 μg g^{-1}. Thus, addition of abiotic or bacterially-produced nano-Se^0 to mercury-contaminated soil under anaerobic and aerobic conditions led to a decrease in the proportion of Hg^0 present, and an increase in the insoluble residual Hg fraction.

The efficiency of abiotic nano-Se^0 preparations was less than that for biogenic nano-Se^0 after SDS treatment which is surprising since the diameter of the abiotic nano-Se^0 (10-90 nm) was much smaller than that of biogenic nano-Se^0 (200-800 nm). In general terms, selenium capture of mercury occurs by a gas-solid reaction where the capacities and kinetics mainly depend on surface area: smaller particles have a larger specific surface area (Johnson et al., 2008). However, the PVA template may have blocked some elemental mercury access to elemental selenium which would

inhibit the reaction between selenium and mercury. Other workers have found similar results, e.g. BSA-stabilized nano-Se^0 had a lower sorption capacity than conventional selenium powder despite a much smaller particle size (6-60 nm vs 10-200 μm) (Johnson et al., 2008). Thus, biogenic nano-Se^0 gave a better performance for Hg^0 immobilization in soil.

3.4 Speciation of Hg immobilized in soil

According to the SEM-EDS of immobilization products (Fig. 5), the atomic ratio of Hg:Se is close to 1 (Table 1), which revealed the formation of HgSe. XRD (Fig. 6) also confirmed that mercury and selenium were in the form of HgSe (PDF#65-2892). XPS analysis shows that binding energy of Hg $4f_{7/2}$ and Hg $4f_{5/2}$ was observed at 99.2 eV and 104.3 eV, respectively (Fig. 7), indicating that mercury could be present as HgSe and HgO (Zylberajch-Antoine et al., 1991). Deconvolution of the high resolution XPS spectra of selenium shows the presence of binding energy peaks of Se $3d_{5/2}$ at 53.8 eV and Se $3d_{3/2}$ at 54.5 eV, which is in good agreement with that previously reported for HgSe (Wall et al., 1986). The binding energy values for Se^0 at Se $3d_{5/2}$ (54.7 eV) and Se $3d_{3/2}$ (55.2 eV) were in accordance with those reported in the literature (Miyake et al., 1984). The XPS results confirmed immobilization of Hg^0 by nano-Se^0 as HgSe.

To date, many technologies have been examined for remediation of mercury-contaminated soil, such as immobilization (stabilization or solidification)

electro-remediation, soil flushing and soil washing, vitrification, thermal desorption and phytoremediation (Wang et al., 2012). Thermal treatment is the most widely used method although the treated soil is unsuitable for reuse due to the destruction of original soil properties (Yang et al., 2008), with some techniques also leading to mercury release into the air (Wang et al., 2012). The work presented here has shown that amorphous nano-Se^0 is capable of capturing Hg^0 in both surface and subsurface soil, thereby reducing mobility due to the production of HgSe. HgS is the typical residual form of mercury in soil, but HgSe also has a very low solubility ($K_{sp}=10^{-58}$) and is more stable than HgS (Björnberg et al., 1988). As well as this, the use of nano-Se^0 appears safer since HgSe is chemically inert and a much less toxic compound compared to other forms of mercury and selenium. In addition, nano-Se^0 is unharmful, and the median lethal dose (LD50) for nano-Se^0 is 6.7 g kg^{-1} in rats (Cummins and Kimura, 1971). Therefore, immobilization of mercury with nano-Se^0 may provide an efficient means of soil remediation with no secondary pollution and no volatilization. However, soil is a heterogeneous complex environment, and it is necessary to consider the wider applicability of this technique across different soil types and physico-chemical conditions as well as the stability of nano-Se^0. Moreover, the effects of different soil compositions and conditions on the reaction between nano-Se^0 and Hg^0 also need to be taken into consideration.

4. Conclusions

This work is the first demonstration that amorphous nano-Se^0 can be applied to

capture Hg^0 in soil under both aerobic and anaerobic conditions. It is concluded that *C. freundii* could be more easily and successfully applied for remediation of surface and subsurface soils, where the redox potential often changes dramatically. The experiments have revealed the formation of non-reactive HgSe by amalgamation of elemental selenium and elemental mercury which provides a potential approach for mercury immobilization in mercury-contaminated sites.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Table 1. The atom concentrations of selenium, mercury and the atomic ratio between selenium and mercury in mercury-contaminated soil after addition of elemental selenium.

	1	2	3	4	5	6	7	8
Atom.C: Hg [at.%]	24.11	19.63	26.09	21.53	24.62	25.89	25.51	28.02
Atom.C: Se [at.%]	21.99	22.02	23.87	20.52	22.15	23.98	22.59	23.8
Atomic ratio Hg/Se	1.096	0.891	1.093	1.049	1.111	1.080	1.129	1.177

As shown in Fig. 5(a), eight points were selected for EDS, and the concentrations of selenium, mercury and the atomic ratio between selenium and mercury were calculated according to the EDS results.

Figure legends

Fig. 1. Growth and reduction kinetics at an initial concentration of 1 mM Na_2SeO_3. Symbols represent: (■) selenite concentration under anaerobic conditions; (□) selenite concentration under aerobic conditions; (▼) CFU under a CFU under aerobic conditions. Error bars (n=3) represent the standard deviation.

Fig. 2. Characterization of elemental selenium produced by *C. freundii* Y9. (a) SEM micrographs of *C. freundii* Y9. (b, c, d) SEM micrographs of *C. freundii* Y9 grown in the presence of 1 mM selenite for 5 d. (e) EDS spectrum and (f) XRD pattern of red selenium particles produced by *C. freundii* Y9 grown in the presence of 1 mM selenite for 5 d. (g, h) Transmission electron micrographs of the cells cultured in 1 mM selenite for 5 d. Scale bars: (a, b) 1 μm; (c, d, g, h) 200 nm. Typical results are shown from one of several determinations.

Fig. 3. SEM micrograph of abiotic elemental selenium. Scale bars = 1 μm. A typical micrograph is shown from one of several determinations.

Fig. 4. Different fractions of mercury in soil before and after experimental treatments. (a) anaerobic conditions. (b) aerobic conditions. Symbols represent: ▨ total mercury; ■ elemental mercury; ▧ water soluble and exchangeable mercury; ▦ mercury bound to organic matter; ▭ residual mercury. Error bars (n=3) represent the standard deviations.

Fig. 5. SEM-EDS micrograph of mercury contaminated soil after addition of elemental selenium. (a) SEM micrograph (scale bar = 2 μm) and (b) EDS spectrum of mercury contaminated soil after addition of elemental selenium. Typical results are shown from one of several determinations.

Fig. 6. XRD pattern of mercury contaminated soil after addition of elemental selenium. (a) XRD pattern of mercury contaminated soil. (b) XRD pattern of mercury contaminated soil with addition of elemental selenium. A typical pattern is shown from one of two determinations both of which gave similar results.

Fig.7. High resolution XPS spectrum. (a) XPS spectroscopy of Hg 4f in experimental soil; (b) XPS spectroscopy of Se 3d in experimental soil. Symbols represent: …… experimental spectrum; — interpolate spectrum; — fitted peaks; — loss feature; — background.